

Occurrence of Proteases in the Salivary Glands of Cocoa-capsids (Heteroptera: Miridae)

R. KUMAR

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF GHANA, LEGON, GHANA

RECEIVED FOR PUBLICATION JANUARY 26, 1970

Abstract: The presence of proteases has been demonstrated in the salivary glands of 5th instar larvae and the adults of mirid pests of cocoa in West Africa. Their significance is discussed.

The occurrence of proteases has been demonstrated in many insects. They consist of a proteinase of the tryptic type for initial breakdown and peptidase or peptidases for final breakdown of polypeptides into amino-acids.

During a review of literature on the enzymes of cocoa-capsids, the reported absence of any proteolytic enzymes in the salivary secretions (Goodchild, 1952) appeared surprising since at least two species (*Helopeltis* and *Bryocoropsis*) are exclusively pod feeders and salivary proteases have been reported in a number of mirids (Nuorteva, 1954).

MATERIAL AND METHODS

Four species of bryocorine mirids, all pests on cocoa in Ghana, were examined; only field collected material being used. The presence of proteases was detected by the method of Pickford and Dorris (1934). The salivary glands from living bugs were dissected out in distilled water, freed from adhering structures (usually not all tracheae could be removed) and placed in a droplet of buffer solution on the gelatin film of a photographic plate. The latter consisted of a negative plate (22DIN-125 ASA) developed in darkness in 20% sodium thiosulphate solution. The plates were left for 12–24 hours at 22°C in a tank saturated with water vapour to prevent evaporation. A droplet of toluene was added during each test to prevent bacterial activity. Buffer drops were used as controls. The plates were stained with acid fuschin which revealed a clear spot at the site of enzyme action. At least two replicates of the test at each pH were performed. Over 600 tests were conducted.

RESULTS

Helopeltis corbisieri Schmitz, *Bryocoropsis laticollis* Schumacher

The pH values were: 4.0, 4.6, 5.0, 5.6, 6.0, 6.6, 7.2, 7.6, 8.0.

Acknowledgments: The author wishes to thank the International Capsid Research Team, Tafo for the supply of photographic plates and the Director, Cocoa Research Institute for the supply of bugs. The author is grateful to Mr. Dennis Leston for comments on the typescript and for his interest in this project.

There was no activity in instars 1–4; the activity was well marked and clear throughout the above range in 5th instar larvae and adults of both sexes. The activity appeared to be slightly greater in the acidic medium.

Sahlbergella singularis Haglund, *Distantiella theobroma* (Distant)

The pH values used were: 4.16, 4.83, 5.0, 5.57, 5.8, 6.0, 6.6, 7.2, 7.6, 8.0.

There was no activity in instars 1–4; there was activity in the 5th instar larvae and adults of both sexes at pH values of 6.0, 6.6, 7.2, 7.6, and 8.0. However, in some experiments on 5th instar larvae of *Distantiella*, slight activity at 5.6 pH was noticed. In several plates of 5th instars and adults of both species there was, for unexplained reasons, no activity throughout the above range.

DISCUSSION

The above results show the presence of proteolytic enzymes in the salivary glands of cocoa-capsids and indicate that several different types of proteases are involved. *Helopeltis* and *Bryocoropsis* have two different types, one active in the acid range and the other in the alkaline range. Probably only a single enzyme is present in *Sahlbergella* and *Distantiella* and possibly this is different from those of *Helopeltis* and *Bryocoropsis*. It is noteworthy that the salivary proteases appear only in the fifth instar and in the adults and it would be interesting to relate the occurrence of these enzymes to the distribution patterns of amino-acids in the haemolymph of larval and adult cocoa-capsids.

The absence of proteases in salivary glands of cocoa-capsids reported by Goodchild (1952) may be due to his use of less sensitive methods. The Pickford and Dorris method used above is capable of detecting proteases at 1 part in 6000. Goodchild (1952) however, reports very slight activity when testing for proteinase in the salivary glands.

Distantiella and *Sahlbergella* differ from *Helopeltis* and *Bryocoropsis* in that they may develop without reduction in fecundity, entirely on green vegetative tissues (Gibbs, Picket and Leston, 1968). It is likely, therefore, that the variable results with fifth instars and adults indicated a heterogeneous sampling population. As a corollary to this it is suggested that there is an inductive mechanism at work. This would have adaptive value since bugs feeding on pods switching over to the production of proteases by salivary glands would be able to penetrate the pod and utilize the proteins. No explanation is suggested by the life-histories (Leston, 1970) concerning absence of production of proteases in the first four instars, even in the two species confined to pod feeding. Gibbs and Picket (1966) noted, however, that in *Distantiella*, under all conditions of water stress, later instars confined on seedlings migrated to the upper, leafy part of the stem whereas instars 1 and 2 were able to exploit the lower, woody part. The scoring classes of these authors however, do not indicate if there is a critical point between fourth and fifth instars.

Perhaps it is possible that the production of proteases by 5th instar larvae and adults is associated with the increased need for amino-acids during the later stages of development of gonads in both sexes.

Literature Cited

- GIBBS, D. G. AND PICKETT, A. D. 1966. Feeding by *Distantiella theobroma* (Dist.) (Heteroptera: Miridae) on Cocoa. I. The effects of water stress in the plant. Bull. Entomol. Res. **57**: 159-169.
- , ———, AND LESTON, D. 1968. Seasonal population changes in Cocoa-capsids (Hemiptera: Miridae) in Ghana. Bull. Entomol. Res. **58**: 279-293.
- GOODCHILD, A. J. P. 1952. A study of the digestive system of the West African Cocoa capsid bugs (Hemiptera: Miridae). Proc. Zool. Soc. Lond., **122**: 543-572.
- LESTON, D. 1970. Entomology of the Cocoa farm. Ann. Rev. Entomol., **15**: 273-294.
- NUORTEVA, P. 1954. Studies on the salivary enzymes of some bugs injuring wheat kernels. Ann. Entomol. Fenn., **20**: 102-124.
- PICKFORD, G. E. AND DORRIS, F. 1934. Micro-methods for the detection of proteases and amylases. Science, **80**: 317-319.